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The Second SeaWiFS HPLC Analysis Round-Robin Experiment (SeaHARRE-2)

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ABSTRACT

Eight international laboratories specializing in the determination of marine pigment concentrations using high performance liquid chromatography (HPLC) were intercompared using *in situ* samples and a variety of laboratory standards. The field samples were collected primarily from eutrophic waters, although mesotrophic waters were also sampled to create a dynamic range in chlorophyll concentration spanning approximately two orders of magnitude (0.3 -25.8 mg m^{-3}). The intercomparisons were used to establish the following: a) the uncertainties in quantitating individual pigments and higher-order variables (sums, ratios, and indices); b) an evaluation of spectrophotometric versus HPLC uncertainties in the determination of total chlorophyll *a*; and c) the reduction in uncertainties as a result of applying quality assurance (QA) procedures associated with extraction, separation, injection, degradation, detection, calibration, and reporting (particularly limits of detection and quantitation). In addition, the remote sensing requirements for the *in situ* determination of total chlorophyll *a* were investigated to determine whether or not the average uncertainty for this measurement is being satisfied. The culmination of the activity was a validation of the round-robin methodology plus the development of the requirements for validating an individual HPLC method. The validation process includes the measurements required to initially demonstrate a pigment is validated, and the measurements that must be made during sample analysis to confirm a method remains validated. The so-called *performance-based metrics* developed here describe a set of thresholds for a variety of easily-measured parameters with a corresponding set of performance categories. The aggregate set of performance parameters and categories establish a) the overall performance capability of the method, and b) whether or not the capability is consistent with the required accuracy objectives.

PROLOGUE

Whether for biogeochemical studies or ocean color validation activities, high performance liquid chromatography (HPLC) is an established reference technique for the analysis of chlorophyll *a* and associated phytoplankton pigments. The emphasis of HPLC methods in marine studies has also been promoted because the international Joint Global Ocean Flux Study (JGOFS) program recommended HPLC techniques in the determination of chlorophyll *a* (JGOFS 1994) and, more precisely, from 1991, to use the Wright et al. (1991) method. More recently, a set of HPLC protocols for marine pigment analyses were codified into the *Ocean Optics Protocols for Satellite Ocean Color Sensor Validation* (Bidigare and Trees 2000), which have been updated more or less on an annual basis (Bidigare et al. 2002 and 2003), and are hereafter referred to as the Protocols.

As part of the *Productivité des Systèmes Océaniques Pélagiques*† (PROSOPE) JGOFS-France cruise, which occurred from 4 September to 4 October 1999, four laboratories, using four different HPLC methods, participated in an intercomparison exercise based solely on natural (field) samples (Hooker et al. 2000). This exercise was called the first Sea-viewing Wide Field-of-view Sensor (SeaWiFS) HPLC Analysis Round-Robin Experiment (SeaHARRE-1), and the samples were collected over a large gradient of trophic conditions (based on the chlorophyll *a* concentration) ranging from the high productivity (upwelling) conditions off the northwestern coast of Africa (2.2 mg m^{-3}) to the oligotrophic regime of the Ionian Sea (0.045 mg m^{-3}).

Despite the diversity in trophic conditions and HPLC methodologies, the agreement between laboratories during SeaHARRE-1 was approximately 7.0% for total chlorophyll *a*, which is well within the 35% accuracy objective for remote sensing validation purposes (Hooker and Esaias 1993). For other pigments (mainly chemotaxonomic carotenoids), the agreement between methods was 21% on average (ranging from 11.5% for fucoxanthin, to 32.5% for peridinin), and inversely depended on pigment concentration (with large disagreements for pigments whose concentrations were close to the methodological detection limits).

Although every effort was made to make SeaHARRE-1 as complete as possible (e.g., all analyses were based on replicates), there were deficiencies in the work plan. Consequently, a follow-on activity to specifically address recommendations from the first round robin was developed: a) a more concerted effort to sample oligotrophic and eutrophic regimes (from a remote sensing perspective, data from these two concentration levels are also where the most new data are needed); and b) the inclusion of standard pigment samples, so a control data set is available for analysis. The use of standard pigment samples was deemed particularly important, because several sources of uncertainty are best quantified if the concentration of the samples are independently known.

The planning for SeaHARRE-2 coincided with an anticipated field deployment to the onshore and deep water environments of the Benguela upwelling system. With

† Translated as the Productivity of Pelagic Oceanic Systems cruise, which is documented at the following Web address: `http://www.obs-vlfr.fr/jgofs/html/prosope/home.htm`.